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Eulimids (Gastropoda, Eulimidae) on the Sea Cucumber *Holothuria mexicana* (Ludwig, 1875) (Holothuroidea, Holothuriidae) in Belize

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Abstract
This study presents new records for 4 eulimid species (Gastropoda, Eulimidae) including *Melanella eburnea* (Megerle von Mühlfeld, 1824), *Melanella hypsela* (Verrill & Bush, 1900), *Melanella* sp., and *Eulimostraca encalada* Espinosa, Ortea & Magaña, 2006 found on the body wall of the sea cucumber *Holothuria mexicana* in Belize. Three of these records (*M. hypsela*, *M. eburnea*, and *E. encalada*) represent a first for the Western Caribbean ecoregion and close a distributional gap for these species in the Caribbean. The geographic distribution of *M. eburnea* is thereby expanded by ~800 km; *M. hypsela* by ~1,200 km and *E. encalada* by ~970 km in the Tropical Northwestern Atlantic province. This article presents for the first time *H. mexicana* as a new host record for *E. encalada.*

Key words
Caenogastropoda; Vanikoroidea; *Melanella*; *Eulimostraca*; parasitic gastropods; shell morphology; Caribbean fauna.

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Introduction

Sea cucumbers (Echinodermata: Holothuroidea) are benthic marine animals that host many symbionts, e.g. turbellarians, polychaetes, gastropods, bivalves, copepods, crabs and fishes (Eeckhaut et al. 2004, Purcell et al. 2016). Some of the symbionts are known to be commensal (Parmentier et al. 2006) and/or parasitic, the latter usually having clear detrimental effects on their host (Hamel et al. 1999).

Molluscs of the Eulimidae family are estimated to be the second largest family of parasitic gastropods, considering all hosts, that also show the greatest diversity and highest level of morphological adaptation (Warén 1983). Eulimids are parasites of all extant classes of echinoderms with which they associate (Warén 1983). Most eulimids are ectoparasitic that feed on the body fluids of echinoderm hosts by using their long proboscis and radula (when present) to pierce the skin and reach internal organs (Crossland et al. 1993, Warén 1980, 1983), or the coelomic fluid, with no serious consequences to the host (Purcell et al. 2016). The eulimids *Melanella acicula* (Gould, 1849) and *Peasistilifer nitidula* (Pease, 1860), for instance, insert their proboscis into the lacunae of

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the host's body wall, not fully penetrating the body wall, and feed upon the coelomocytes (Warén 1980, 1983). When present, harmful effects of parasitic gastropods occur not only through their feeding activities but also through attachment lesions that can induce galls in the host (Eeckhaut et al. 2004).

Eulimids are obligate symbionts that can be more or less firmly attached to their host; species that are permanently attached to the host usually show a highly modified anatomy (Warén 1983). However, free-living specimens, mainly from the genus *Melanella* Bowdich, 1822 and *Vitreolina* Monterosato, 1884 leave their host on a regular basis, usually retreating under rocks or in crevices, possibly to increase the occasions for mating (Warén 1983). The reproductive strategy of eulimids is closely related to the mode of attachment of the species (Warén 1983). However, how much time or in which period of the life history do these eulimids live free from the host remains unknown.

According to Warén (1983), there are about 14 eulimid genera specialized in parasitizing holothuroids. From these, 9 are strictly endoparasitic, 2 are gall formers, and 3 live as ectoparasites. The last one, an undescribed species of the genus *Melanella*, is known to live as an ecto or endoparasite of holothuroids in the orders Holothuriida (formerly Aspidochirotida) and Dendrochirotida (Warén 1983). Some eulimids are known to be host specific, while others are generalists (Crossland et al. 1993).

The genus *Melanella* consists of ~360 species and has a worldwide distribution (MolluscaBase 2018). *Melanella* spp. are found in a range of benthic zones, including the littoral (e.g. Souza and Pimenta 2017), sub-littoral (e.g. Queiroz et al. 2013, Redfern 2013), infralittoral (Verrill and Bush 1900, Dunker 1875, Megerle von Mühlfeld 1824, Souza and Pimenta 2017), continental shelf and slope (Watson 1883, Barros et al. 2001), down to abyssal depths (Bouchet and Warén 1986); their distributions are similar to that of their holothuroid hosts (Souza and Pimenta 2017).

In the present study, we provide taxonomic remarks and geographic distributions (including extension ranges) for 4 eulimid species, i.e. *M. eburnea* (Megerle von Mühlfeld, 1824), *M. hypsela* (Verrill & Bush, 1900), an undetermined *Melanella*, and *Eulimostraca encalada* Espinosa, Ortea & Magaña, 2006, which were found on the dorsal and ventral surfaces of the sea cucumber *Holothuria mexicana* Ludwig, 1875 in Belize (Central America). The prevalence of eulimid infestation was also assessed.

Methods

A total of 121 individuals of *H. mexicana* were collected in southern Belize (Caribbean Sea) between 2014 and 2016, including 51 in the Placencia Lagoon (16°30′34″ N, 088°22′32″ W) and 70 in the Port Honduras Marine Reserve (PHMR; 16°11′41″ N, 088°37′49″ W). The Placencia Lagoon is characterized by seagrass beds, sand and mud bottom and is surrounded by mangrove vegetation. The site where the holothuroid hosts were found is characterized by seagrass beds and sand bottom. The PHMR contains 4 river estuaries including those of the Monkey River, Deep River, Golden Stream and the Rio Grande in addition to the Ycacos lagoon. In 2014–2015, specimens were collected under Belize Fisheries Department (BFD) Marine Research Permit 000005-14 for the reproduction of sea cucumbers in Belizean waters; in 2016, specimens were collected under a special permit granted by the BFD to collect sea cucumbers for a processing demonstration. Eulimid epibionts, photographed with their hosts in the field and removed from the body surfaces of the sea cucumbers, were preserved in 70% ethanol and subsequently identified based on shell morphology. The number of eulimids on each holothuroid was counted to determine the prevalence, i.e. the ratio of infested to total holothuroid hosts; and the intensity, i.e. the number of eulimid parasites per infested host.

The geographic distribution of each species was compiled from the literature. In the case of *Melanella* species, the data source was the recent review of Souza and Pimenta (2017), whereas for *E. encalada*, different sources were used, such as Redfern (2001) and Espinosa et al. (2006, 2017). The provinces and ecoregions proposed by Spalding et al. (2007) were used for the description of the geographic distributions.

Acronyms of collections where the material was studied and catalogued: Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil (MNRJ); Museo de Zoología, Universidad de Costa Rica, San José, Costa Rica (MZUCR); Natural History Museum, London, United Kingdom (NHMUK); Smithsonian National Museum of Natural History, Washington DC, USA (USNM). Due to the recent fire in MNRJ (Zamudio et al. 2018), the vouchers in this collection were possibly destroyed.

Results

Order Littorinimorpha
Superfamily Vanikoroidea
Family Eulimidae

***Melanella eburnea* (Megerle von Mühlfeld, 1824)**
Figures 1A–D, 4A

Material examined. Belize: Stann Creek, Placencia Lagoon (16°30′34″ N, 088°22′32″ W, 4 m depth), coll. Arlenie Rogers, 13 March 2014, on dorsal body wall surface of *H. mexicana*, MNRJ 29556, 2 specimens.

Identification. Souza and Pimenta (2017) reviewed the taxonomy of this species recently, based on 65 individuals collected mainly in Brazil but also in Caribbean. The original description is brief and the type is unknown (Souza and Pimenta 2017). All specimens of *M. eburnea* collected in Belize had a broken protoconch (Fig. 1A, B), however the outline of the body whorl (Fig. 1C–D) fits the morphology described by Souza and Pimenta

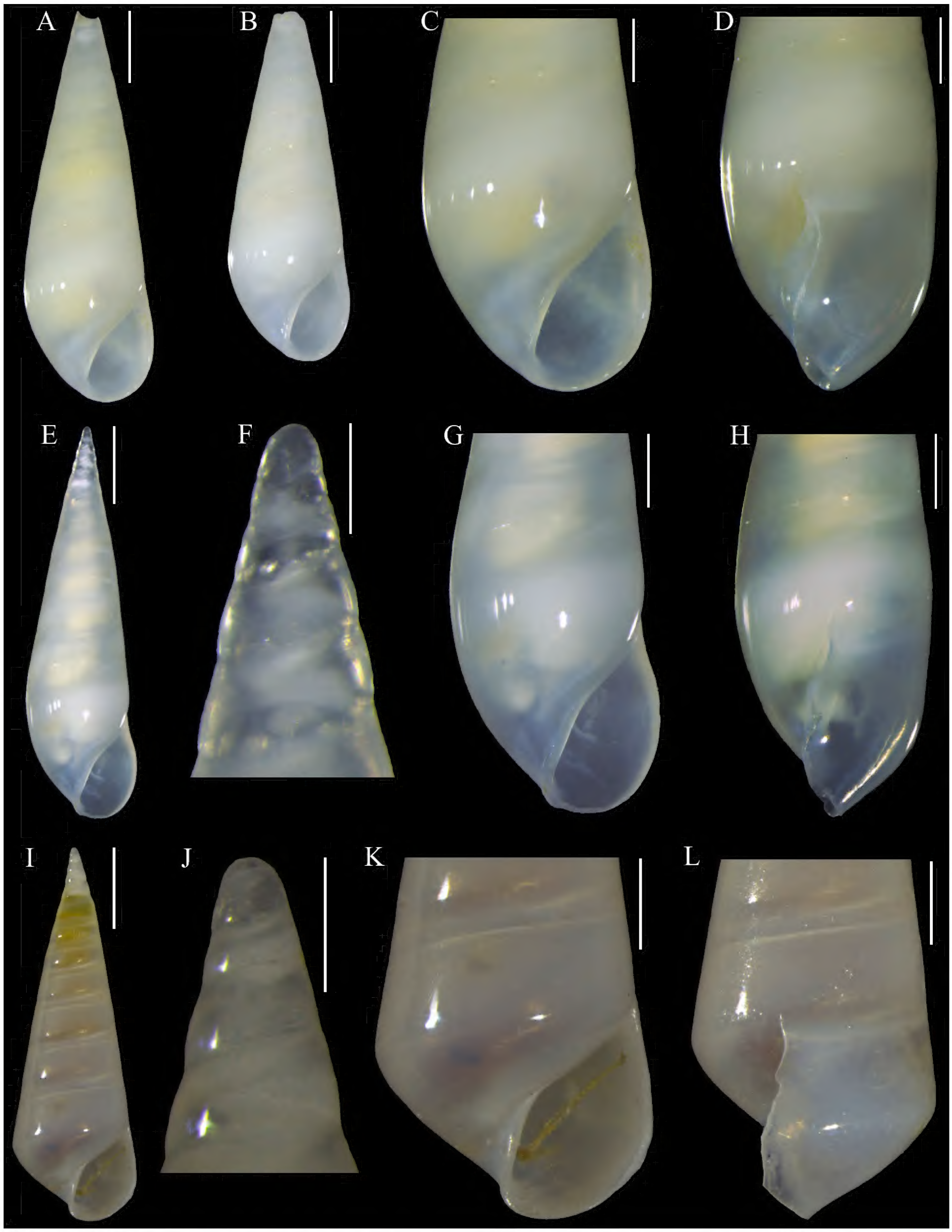

Figure 1. *Melanella* species from Belize. **A–D.** *M. eburnea* (von Mühlfeld, 1824): MNRJ 29556. **E–H.** *M. hypsela* (Verrill & Bush, 1900): MNRJ 29549. **I–L.** *Melanella* sp.: MNRJ 29557. **A–B, E, I.** Shell, frontal view. **C, G, K.** Body whorl, frontal view. **D, H, L.** Body whorl, lateral view. **F, J.** Apical whorls, frontal view. Scale bars: A–B, E, I = 1.0 mm; C–D, G–H, K–L = 0.5 mm; F, J = 0.2 mm.

(2017). The base is rounded, elongated and connected to the anterior region of the aperture (Fig. 1C). *Melanella eburnea* differs from *M. hypsela* (Fig. 1E–H), a similar species occurring in the Western Atlantic, because the latter presents a slightly more elongated aperture, with a base connected close to the anterior region of the aperture rather than at the distal point. Furthermore, the protoconch of *M. eburnea* usually has a flatter outline, while in *M. hypsela*, it is more convex (Souza and Pimenta 2017).

Measurements (mm). Whorls = 8, shell length = 5.43, body whorl length = 2.61, aperture length = 1.67, shell width = 1.80, aperture width = 0.96.

Geographic distribution. Tropical Northwestern Atlantic: Bahamian, Southwestern Caribbean, Eastern Caribbean (Souza and Pimenta 2017), Western Caribbean (this study; ~800 km western extension range); Tropical Southwestern Atlantic: Northeastern Brazil, Eastern Brazil; Warm Temperate Southwestern Atlantic: Southeastern Brazil (Souza and Pimenta 2017).

Infestation prevalence. Of 51 individuals of *H. mexicana* collected at the Placencia Lagoon, 2 hosted 1 individual of *M. eburnea* each, on the dorsal side of the body wall. The prevalence was 0.0392 (presence of parasite in 3.92% of the specimens examined).

***Melanella hypsela* (Verrill & Bush, 1900)**

Figures 1E–H, 4B

Material examined. Belize: Stann Creek, Placencia Lagoon (16°30′34″ N, 088°22′32″ W, 4 m depth), coll. Arlenie Rogers and Jean-François Hamel, 22 August 2016, on dorsal surface of *H. mexicana*, MNRJ 29565, 1 specimen; same locality and host, coll. Arlenie Rogers, 13 March 2014, MNRJ 29549, 1 specimen.

Identification. Souza and Pimenta (2017) reviewed the taxonomy of this species recently, based on the type material and more than 60 individuals from Brazil. The shell morphology of specimens collected in Belize (Fig. 1E–H) agreed completely with the description they provided, presenting a conical protoconch of slightly convex outline (Fig. 1F), a teleoconch with flat outline (Fig. 1E), and a slightly elongated aperture (Fig. 1G).

Measurements (mm). Whorls = 12, SL = 5.04, BWL = 2.11, AL = 1.12, SW = 1.43, AW = 0.83.

Geographic distribution. Warm Temperate Northwest Atlantic: Carolinian, Northern Gulf of Mexico (Souza and Pimenta 2017); Tropical Northwestern Atlantic: Floridian, Bermuda, Bahamian, Greater Antilles (Souza and Pimenta 2017), Western Caribbean (this study; ~1,200 km southwestern extension range), Southern Caribbean (Souza and Pimenta 2017); North Brazil Shelf: Amazonia; Tropical Southwestern Atlantic: Eastern Brazil, Trindade and Martin Vaz Islands (Souza and Pimenta 2017); Warm Temperate Southwestern Atlantic: Southeastern Brazil (Souza and Pimenta 2017).

Infestation prevalence. Of a total of 51 individuals *H. mexicana* collected at the Placencia Lagoon, 2 hosted 1 individual of *M. hypsela* each, on the dorsal side of the body wall. The prevalence was 0.0392 (presence of parasite in 3.92% of the specimens examined).

***Melanella* sp.** (Figs 1I–L, 3A)

Material examined. Belize: Toledo, Port Honduras Marine Reserve (16°11′41″ N, 088°37′49″ W, 8.5 m depth), coll. Arlenie Rogers, 06 February 2015, on dorsal surface of *H. mexicana*, MNRJ 29557, 1 specimen.

Identification. The single specimen (Fig. 1I–L) of this taxon presents a similar protoconch as specimens of *M. hypsela*, however the shell differs by the outline of the base, which is truncated, and by the rhomboid aperture (Fig. 1K). These features are very common in young specimens of several groups of Eulimidae (Lyons 1978, Souza, pers. obs.). Shells of *M. hypsela* with a similar number of whorls examined here and by Souza and Pimenta (2017) do not show the truncated base and rhomboid aperture. This specimen collected in Belize may be an aberrant form of *M. hypsela*.

Another *Melanella* that can be associated with this taxon is *Melanella conoidea* (Kurtz and Stimpson, 1851), based on the outline of the base and aperture. However, the name *M. conoidea* needs clarification, because the original description is brief, has no figures and the type material is missing (Olsson and Harbison 1953). The type material was possibly destroyed in the "Great Chicago Fire", as many other types of William Stimpson deposited in the Chicago Academy of Sciences (Mikkelsen and Bieler 2007). Based on these circumstances, we prefer to keep this provisional identification rather than affirm a specific determination that lacks confidence.

Measurements (mm). Whorls = 11, SL = 4.62, BWL = 1.91, AL = 1.16, SW = 1.53, AW = 0.81.

Geographic distribution. Tropical Northwestern Atlantic: Western Caribbean (this study).

Infestation prevalence. Of a total of 51 individuals of *H. mexicana* collected at the PHMR, only 1 individual hosted 1 individual of *Mellanella* sp., on the ventral surface. The prevalence was 0.0196 (presence of parasite in 1.96% of the specimens examined).

***Eulimostraca encalada* Espinosa, Ortea & Magaña, 2006**

Figures 2A–C, F–J, 3B, 4C

Material examined. Belize: Stann Creek, Placencia Lagoon (16°30′34″ N, 088°22′32″ W, 4 m depth), coll. Arlenie Rogers and Jean-François Hamel, 22 August 2016, on dorsal surface of *H. mexicana*, MNRJ 29561, 1 specimen. Costa Rica, Limón, Gandoca-Manzanillo (09°39′38″ N, 82°39′32″ W, 23 m depth), coll. José Espinosa, 30 September 2003, MZUCR INB 3761771, 2 shells.

Identification. The specimen from Belize (Figs 2A–B, F–G, 3B) does not present a strong coloration even as the shell illustrated by Redfern (2013: fig. 319), but the color pattern and general shape of the shell fits the holotype of *E. encalada* except for the more truncated base (Espinosa et al. 2006: fig. 1H–I). The holotype shell is 3.22 mm long with almost 10 whorls, slightly longer than the specimen from Belize. One specimen from Costa Rica (Fig. 2C, I–J) showing similar dimensions to the individual from Belize

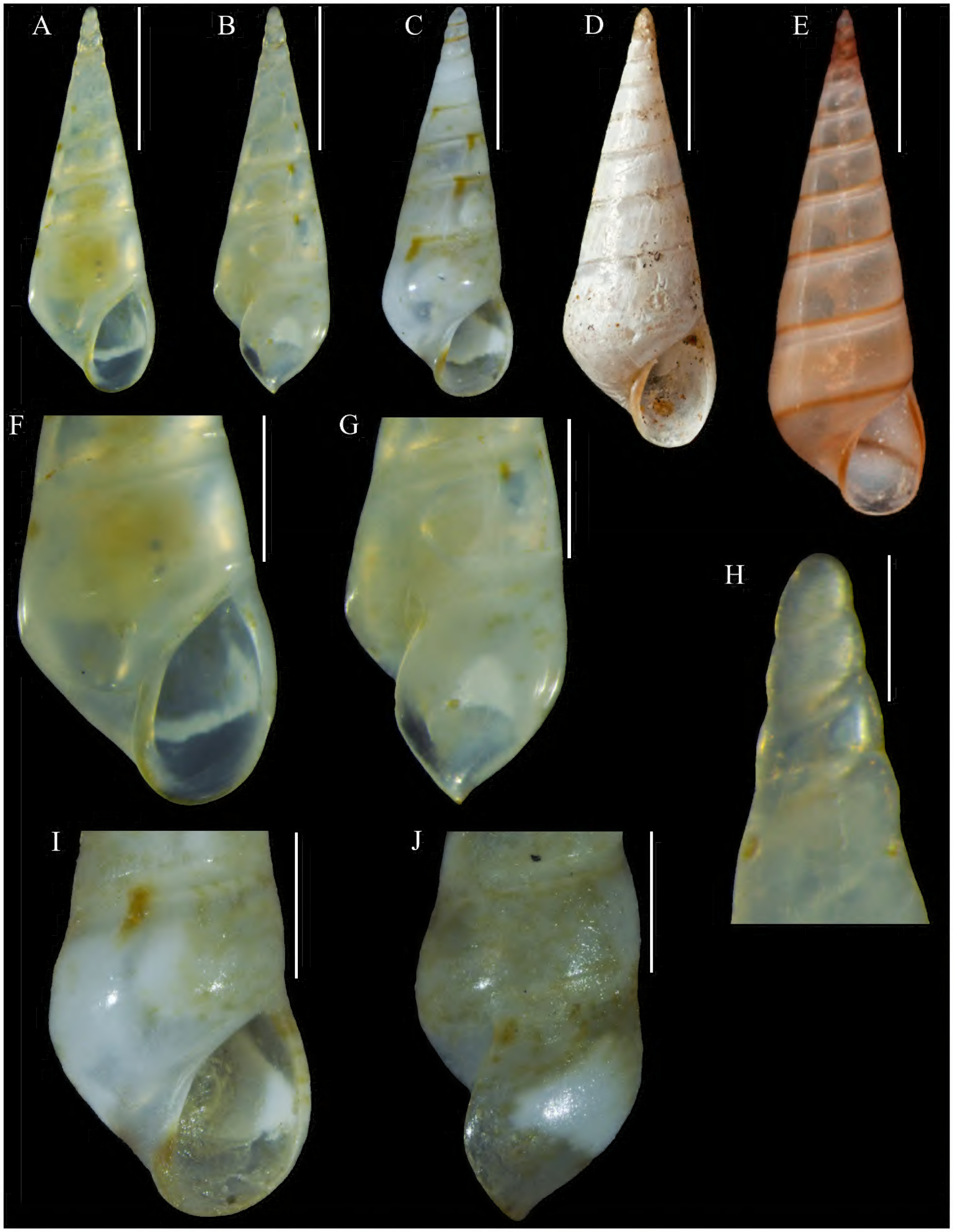

Figure 2. *Eulimostraca* species. **A–C, F–H.** *E. encalada* Espinosa, Ortea & Magaña, 2006: specimen from Belize, MNRJ 29561. **D–E.** *E. subcarinata* (d'Orbigny, 1841): **D.** Holotype of *Eulima subcarinata* d'Orbigny, 1846 (NHMUK 1854.10.4.141); **E.** USNM 875012, off Charleston, South Carolina, USA (33°12'48" N, 78°23'18" W, 35–37 m). **C, I–J.** *E. encalada*: specimen from Costa Rica, MZUCR INB 3761771. **A, C–E.** Shell, frontal view. **B.** Shell, lateral view. **F, I.** Body whorl, frontal view. **G, J.** Body whorl, lateral view. **H.** Apical whorls, frontal view. Scale bars: A–E = 1.0 mm; F–G, I–J = 0.5 mm; H = 0.2 mm. Image 2D courtesy of Harry Taylor, NHMUK Photographic Unit.

is illustrated here for comparison. Both specimens have brownish spiral lines in the protoconch but are lighter in the specimen from Belize (Fig 2H), brownish axial bands in the incremental scars (Fig. 2A–C) and in the inner lip (Fig. 2F, I), small brownish blots in the teleoconch surface (Fig. 2F–G, I–J), and a pointed protoconch with

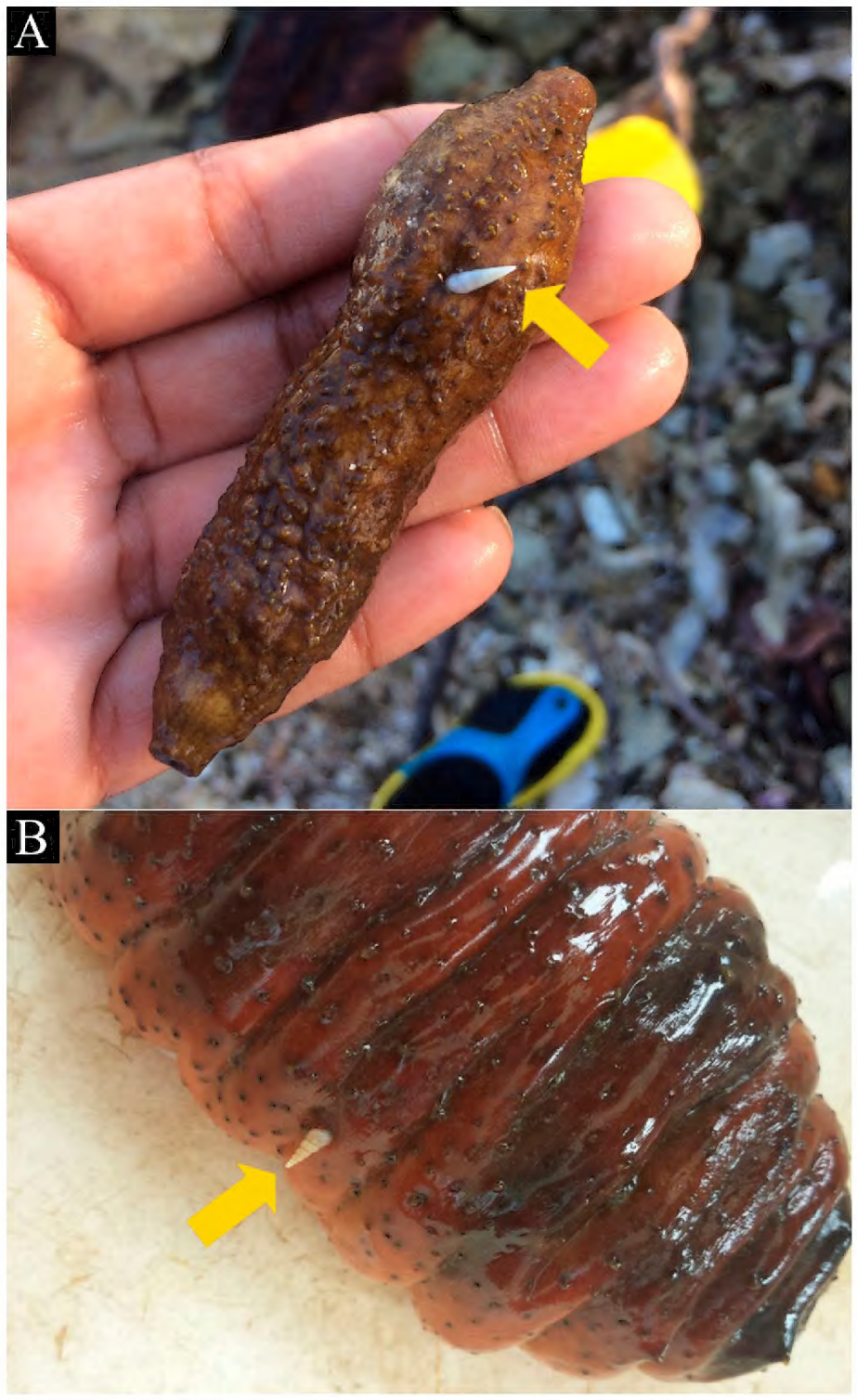

Figure 3. Eulimidae attached to the dorsal surface of *H. mexicana* in Belize. **A.** *Melanella* sp., at Port Honduras Marine Reserve. **B.** *E. encalada*, at Placencia Lagoon.

relatively convex whorls (Fig. 2C, H). The dimensions are also very similar; both present around 9.0 whorls and a length between 2.7–2.8 mm.

Eulimostraca subcarinata (d'Orbigny, 1841), known from a wide range in the western Atlantic (Rosenberg et al. 2009), is a similar species in shape and size, but has a different color pattern. Lyons (1978) redescribed this species and illustrated the holotype NHMUK 1854.10.4.141, which is also illustrated here (Fig. 2D). The holotype is severely corroded, but has brownish spiral line close to suture in the teleoconch and a truncated base (d'Orbigny 1841, Lyons 1978). The latter feature is variable as described by Lyons (1978) who examined several specimens with a more rounded base (Fig. 2E). This variation can also be observed in *E. encalada* (Fig. 2A, C). In addition to the presence of a brownish spiral line, well-preserved specimens of *E. subcarinata* have a darker brown protoconch and teleoconch surface and lacks axial bands and irregular blots (Fig. 2E) (Lyons 1978).

Measurements (mm). Whorls = 9, SL = 2.70, BWL = 1.30, AL = 0.74, SW = 0.89, AW = 0.50.

Geographic distribution. Tropical Northwestern Atlantic province: Bahamian (Espinosa et al. 2006, Redfern 2001), Greater Antilles (Espinosa et al. 2006, Espinosa et al. 2017), Western Caribbean (this study; ~970 km southwestern extension range), Southwestern Caribbean (Espinosa et al. 2006).

Infestation prevalence. From a total of 121 individuals of *H. mexicana* collected at the Placencia Lagoon, only 1 hosted 1 individual of *E. encalada*, on the dorsal surface (Fig. 22). The prevalence was 0.0196 (presence of parasite in 1.96% of the specimens examined).

Discussion

Melanella eburnea, *M. hypsela* and *E. encalada* are reported for the first time in the Western Caribbean ecoregion (Fig. 4A–C), thereby expanding their geographic distributions by ~800 km, ~1,200 km and ~970 km, respectively, from previous records in the Tropical Northwestern Atlantic province. Both species of *Melanella* have a wide geographic distribution in the Tropical Atlantic and Temperate South America realms; *M. hypsela* occurs from Bermuda to the southeast coast of Brazil (Fig. 4B) and *M. eburnea* occurs from the Bahamas to the south coast of Brazil (Fig. 4A). *Melanella eburnea* has doubtful records in the Eastern Atlantic at Cape Verde and Ascension Island (Brown et al. 2016) as discussed by Souza and Pimenta (2017). The records in Belize closes a distributional gap for these species in the Caribbean (Figs 4A–C). *Eulimostraca encalada* has a more restricted distribution, known only from the Tropical Northwestern Atlantic province (Fig. 4C). *Melanella eburnea* and *M. hypsela* were previously recorded parasitizing *H. mexicana* in the Caribbean by Warén (1983) and Souza and Pimenta (2017).

Species of *Eulimostraca* possess a radula (Warén 1983), but the mode of feeding was previously unknown, and an echinoderm host had never been reported (Warén 1992), except for a doubtful identification by Sonnenholzner and Molina (2005), who recorded *Eulimostraca burragei* (Bartsch, 1917) (as *Balcis panamensis*) parasitizing 2 species of holothuroids: *Holothuria portovallartensis* (Caso, 1954) and *Holothuria theeli* (Deichmann, 1938). However, the specimen illustrated by Sonnenholzner and Molina (2005) has a broken protoconch; therefore we cannot be certain whether the specimen really belongs to *Eulimostraca*.

As the generic classification of the species reported by Sonnenholzner and Molina (2005) is dubious, the record of *E. encalada* attached to the dorsal surface of the sea cucumber *H. mexicana* (Fig. 3B) may be the first record of a host-parasite relationship in *Eulimostraca*. Warén's (1983) hypothesis suggests that a genus of Eulimidae usually parasitizes an exclusive class or a lower taxon of Echinodermata. During sampling (Fig. 3B), there was a small scar on the body of the holothuroid after each eulimid was removed, although there was no evidence

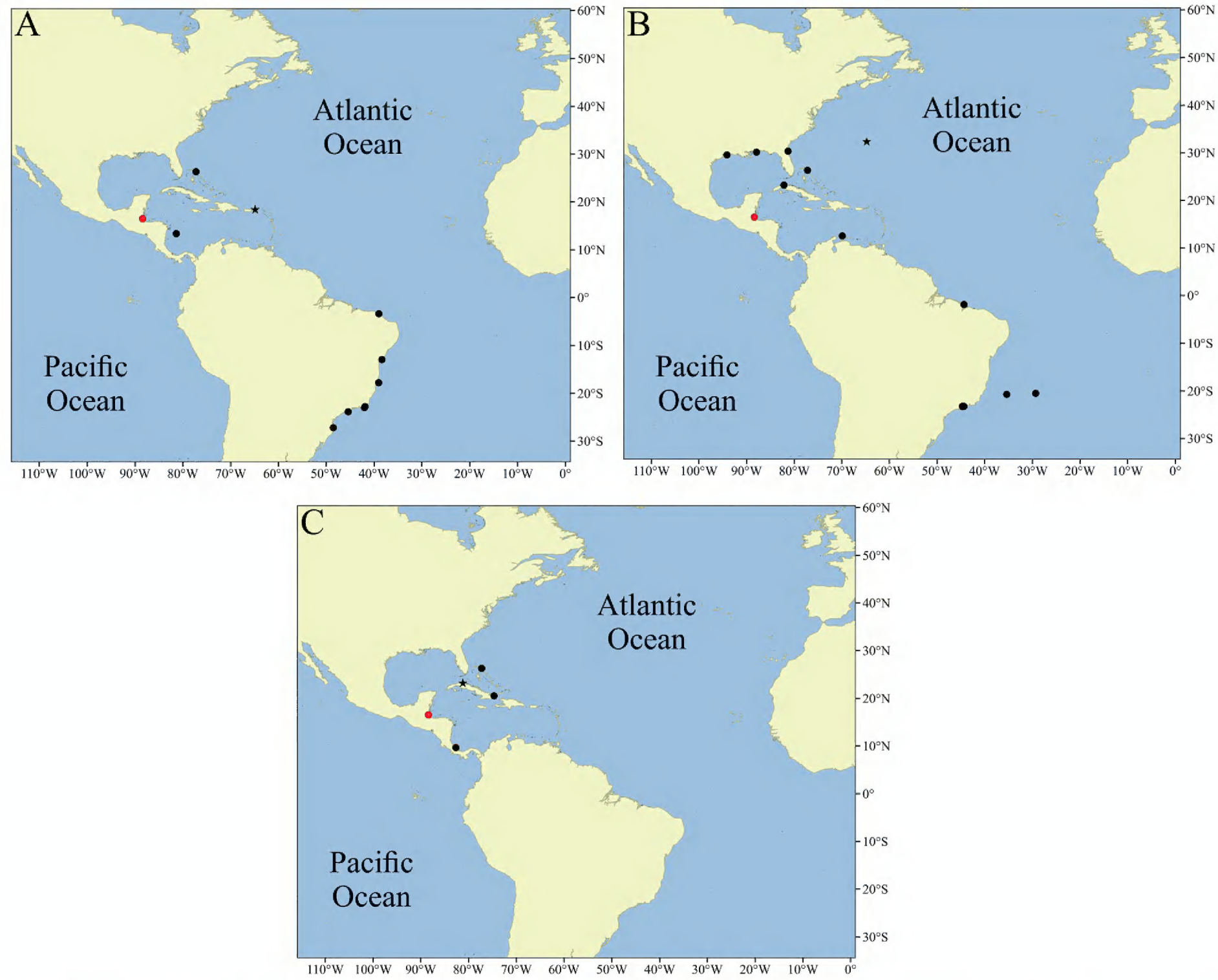

Figure 4. Geographic distributions of the species collected in Belize based on confirmed records. **A.** *M. eburnea*. **B.** *M. hypsela*. **C.** *E. encalada*. Star = type locality; black dots = literature records; red dots = new records (this study).

of a proboscis penetrating the holothuroid, the method of feeding in most eulimids (Warén 1983), but the specimen of *E. encalada* was attached to the surface of *H. mexicana* suggesting that species of this genus are possible parasites of Holothuroidea. However, more data about the biology of *Eulimostraca* species would be required to clarify the parasitic strategy or intimacy of the relationship with the host in this group of gastropods. For *E. encalada*, the sea cucumber *H. mexicana* represents a new host record (Fig. 3B).

In the present study, the infestation prevalence was low for all 4 eulimid species (1.96 and 3.92%). Records of Eulimidae associated (as endoparasites or ectoparasites) with holothuroids are being more frequently reported in the literature (e.g. Delongueville and Scaillet 2009, Goto 2010, Queiroz et al. 2013, Delongueville and Scaillet 2015, Nekhaev 2016, Souza and Pimenta 2017, Takano et al. 2017), but most of them are qualitative, presenting which species are involved with few mentions of infestation prevalence. Caso (1968) reported "*Balcis intermedia* (Cantraine, 1835)" as an ectoparasite of *Holothuria glaberrima* Selenka, 1867 in Veracruz, Mexico with a high prevalence of about 60%. The name *Eulima intermedia* Cantraine, 1835 is an invalid name (rejected by ICZN Opinion 1780, 1994) and was applied to an assortment of species (Souza and Pimenta 2017). Despite the good schematic figures presented by Caso (1968), the identification of the species is hard but the latter author possibly reported *M. eburnea* or *M. hypsela*. Sonnenholzner and Molina (2005) reported the ectoparasite "*Balcis panamensis*" in the Galapagos Islands, Ecuador, associated to *H. portovallartensis* and *H. theeli* with high prevalences of 95% and 79%, respectively. Up to 8 individuals of "*B. panamensis*" were found in a single individual of *H. portovallartensis*.

Mohan and James (2005) reported an infestation prevalence of about 0.1% for *Megadenus* sp. (endoparasitic) on *Holothuria atra* Jaeger, 1833. Takano et al. (2017) found more populations of the eulimid reported by Mohan and James (2005), which was described as *Megadenus atrae* (Takano et al. 2017), and is one of the most detailed investigations reporting the prevalence of eulimid parasites among hosts. Takano et al. (2017) reported infestation prevalences varying from 0 to 10% in 17 populations of *H. atra* in the West Pacific (New Caledonia and Japan); the authors examined a total of

3,848 holothuroids and found 66 specimens of *M. atrae* (overall infestation rate of 1.7%).

While they appear to be geographically widespread, Eulimidae are rarely found in great numbers in association with their hosts, hampering studies on their life history (Matsuda et al. 2012). The collection of more specimens would provide opportunities for laboratory experiments (e.g. Will 2009, Dgebuadze and Kantor 2016), help increase biological and ecological knowledge (e.g. population dynamics, growth, reproduction), and ultimately clarify parasitic strategies and the degree of dependence on the hosts.

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Authors' Contributions

AR and JFH collected holothuroids and eulimids, and took the photographs in the field; LSS identified the species, measured the specimens and took the photographs in the laboratory; all authors wrote the manuscript.

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